



**S/N 09/785546**

**PATENT**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Sonya Franklin

Examiner: Charles L. Patterson, Jr

Serial No.: 09/785546

Group Art Unit: 1652

Filed: February 16, 2001

Docket No.: 875.037US1

Title: ARTIFICIAL ENDONUCLEASE

**DECLARATION UNDER 37 C.F.R. § 1.132**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

I, Dr. Sonya Franklin, declare as follows:

1. I am the inventor of the claims of the present application and make this Declaration in support of the patentability of the claims of the above-referenced application.
2. In the Office Action dated March 17, 2004, the Examiner questions whether Figure 8 in the specification shows that complexes of a chimeric peptide of the invention and a metal can convert supercoiled DNA to open circular and/or linear DNA.
3. Various combinations of supercoiled plasmid DNA, metal and peptide were mixed and incubated, and the relative amounts of supercoiled, open circular and/or linear forms of DNA for each combination were detected by agarose gel electrophoresis. As described in the specification, lane 1 in Figure 8 contains pUC19 plasmid DNA, a negative control. Lanes 2-5 in Figure 8 contain plasmid DNA with increasing concentrations of  $\text{EuCl}_3$ . It is well known that certain metals will hydrolyze DNA (see page 2, lines 5-6 of the specification) and the data in lanes 2-5 confirm that Eu cleaves supercoiled DNA. Lanes 6-9 in Figure 8 contain plasmid DNA plus increasing concentrations of free chimeric peptide

- P3. Although it may appear that P3 cleaves DNA, the observed results were not significant relative to controls. Lanes 10-15 in Figure 8 contain plasmid DNA plus increasing concentrations of EuP<sub>3</sub>. As noted at page 56, lines 14-16 of the specification, the highest amounts of nicking of DNA by EuP<sub>3</sub> occurred at intermediate concentrations as at higher concentrations of EuP<sub>3</sub> (lanes 13-15), significant affinity for DNA resulting in precipitation decreased apparent total DNA intensity. Figure 8 thus shows that EuP<sub>3</sub> cleaves supercoiled DNA.
4. The concentration dependent formation of open circular plasmid from supercoiled plasmid DNA in the presence of P3-metal complexes is also shown in Figure 3 of Welch et al. (J. Inorg. Chem., 40:1982 (2001), reproduced in the right panel of Figure 1 enclosed herewith, as well as in results from a further experiment which are shown in the left panel of Figure 1. These results confirm that EuP<sub>3</sub> cleaves supercoiled DNA at low concentrations.
  5. Similar results were observed with another chimeric peptide, P4, as reported in Sirish et al. (J. Inorg. Biochem., 91:253 (2002), of record). In particular, Sirish et al. report that conversion of supercoiled to nicked or linear DNA was greater in the presence of P4-metal complexes than in controls.
  6. Thus, peptides of the invention complexed with a metal cleave supercoiled DNA.

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7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.


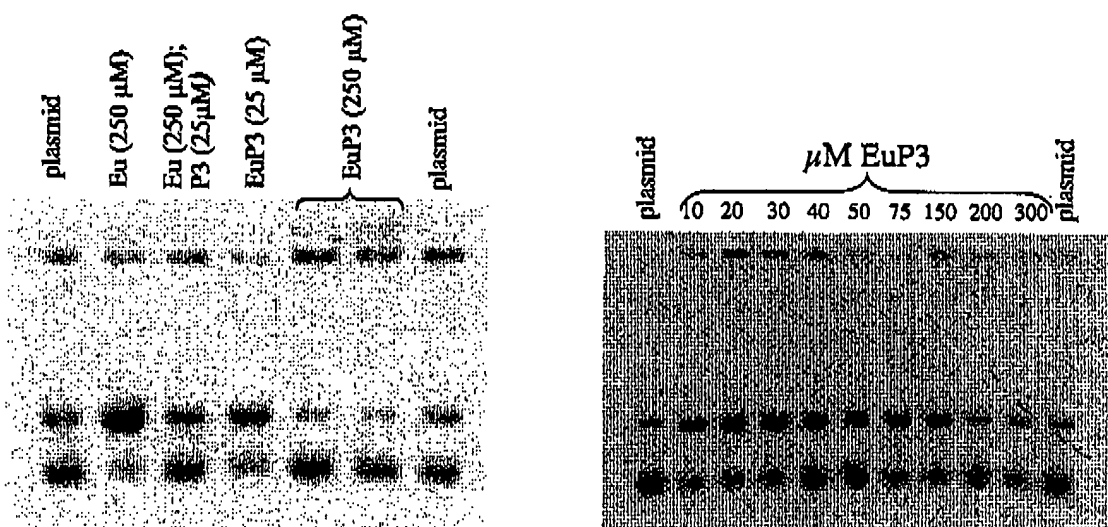
Dated: 6/28/04By:   
Dr. Sonya Franklin

Figure 1. EuP3 cleaves supercoiled DNA.



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